July 5, 1954

Dear Professor Hinshelwood:

I have been struck by the degree to which current controversies on the mechanism of bacterial adaptation may be at cross-purposes owing to the divergence of experimental material, a situation that might be readily corrected. If I may take the liberty of the remark, your observations on the adaptation of B. lactis aerogenes to the utilization of D-arabinose represent the clearest support of your arguments; the multistep variations involved in resistance to proflavine would require a much more elaborate review. At any rate, if I can find the time, I would like the opportunity of reviewing the situation on my own laboratory bench— particularly the experiment represented by figure 1 in the paper by Baskett and yourself, PRS,B139:58. May I ask your assistance in furnishing the strain you used for these experiments? To avoid any confusion, I should also like to have for comparison a subculture of what you would certify to be an irreversibly "trained" strain.

The S.E.B. issue on Evolution has just come to our library, and I was most pleased to see the clarity with which you presented the issue (though I will not pretend that your argument and conclusion are such that I can fully acquiesce in them), especially at page 32, that we are concerned at the means of irreversibility. Noone has questioned that physiological adaptations occur, nor that they are represented in your experiments, but this very fact tends to confuse the experimental decision. In most of your work, my attempted interpretation (as you know) would be that induced physiological adaptations had permitted the development of populations large enough that spontaneous variations might then occur and be selectively fixed, a mechanism hardly distinguishable from Waddingtons findings on Drosophila (at pp.194-198 of the same symposium). I would not argue that genetic factors are required by natural law to be so insulated from the day to day history of the cell; but my reading of the evidence is that this is what happens to have come about during the evolution of living forms. I can assure you that I would be quite prepared to entertain evidence to the contrary, but so far (with some tortuosity to be sure!) the mutation theory does not seem to me to have failed. However, I could comment on this with less prejudice if I could reexamine relevant material with my own hand.

May I take the occasion to renew my request for reprints, a favor I am happy to reciprocate. I lack the following that have appeared in the Proc. Roy. Soc.: (Dean and Hinshelwood) 1952 140:339; (Hinshelwood and Jackson) 137:88; 136:562; and(Kilkenny and Hinshelwood) 139:575, in addition that others that may have appeared subsequently.

I have noted your correction in Nature as to your "disregard" of selection mechanism. If "Bacterial Physiology" could be revised, I would rewrite this chapter to fit more closely to your current views; I have had an opportunity to substitute "minimized" in later printings, which I hope does not effect too much of a distortion. I should have quoted your letter of 16 Feb 1949 in the wording "to explore the potentialities" in place of "to bolster the applicability": perhaps I was influenced by your paper with Peacocke(1948) which seemed, in a very different spirit (and to my mind wholly without justification) to deny the materiality of the auxotrophic mutants that are the daily utensils of microbial genetics! However, your subsequent writings, including the letter to Nature seem to have adopted a "more eclectic outlook", so I trust there need be no further quarrel. By the way, you do me too much honor in attributing "Bacterial Physiology" to my authorship.

I do not have the final corrected volume, but the proofs of the S.G.M. symposium of last year contained in line (your paper with Dean, the terminal

paragraph)
that was entirely mystifying— perhaps you would be kind enough to clarify it. "A synthetic agar plate was spread with 2 x 10^7 [sic] cells.... single colonies were ~~spread~~ visible on this plate". Can you distinguish so many single colonies on a plate, or is the figure a typographical error? If so few cells were inoculated that single colonies ~~were~~/ developed, the experiment is indecisive (from the selectionist viewpoint) since any mutants transferred to the replica plate must have constituted a negligible proportion of the colony during whose growth they must have arisen. If there were 2×10^7 colonies (which I suppose could be distinguished under the microscope), I don't see how one could maintain so precise a correspondence, after two replicas, that one could expect congruence by a factor of $5/2 \times 10^7$, that is a resolution of this fraction x the area of a Petri dish, =75 cm^2, or about $(0.4\ mm)^2$. But even accepting this technical tour de force, the next plating suggests that these 5 colonies altogether had less than 1% mutant cells, which is quite compatible with the possibility of a mutant having arisen some time after the 16-32 cell stage of any of the colonies. The later history of the single colony of the 100 whose replica did show a resistant shows that this colony did not come from a mutant cell, but that a new mutation had occurred during its development. I can assure you that had I followed a similar protocol, I would not have obtained indirectly selected streptomycin-resistant mutants either. The maximum enrichment that we found could be expected in practice was about 100-fold at each stage, and this has to be followed by sample platings to be sure one has recovered the mutant clone, unless relatively large areas are re-picked from the plates.

Yours sincerely,

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